UNIVERSITI PUTRA MALAYSIA

GENERATION, PHENOTYPING AND FUNCTIONAL ANALYSIS OF DENDRITIC CELLS PC) DERIVED FROM HUMAN MONOCYTES AND ACUTE MYELOID LEUKAEMIA (AML) CELLS

LIM MOON NIAN

FPSK(M) 2004 2

GENERATION, PHENOTYPING AND FUNCTIONAL ANALYSIS OF DENDRITIC CELLS (DC) DERIVED FROM HUMAN MONOCYTES AND ACUTE MYELOID LEUKAEMIA (AML) CELLS

By

LIM MOON NIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2004

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

GENERATION, PHENOTYPING AND FUNCTIONAL ANALYSIS OF DENDRITIC CELLS (DC) DERIVED FROM HUMAN MONOCYTES AND ACUTE MYELOID LEUKAEMIA (AML) CELLS

By

LIM MOON NIAN

June 2004

Chairman: **Professor Seow Heng Fong, Ph.D.**

Faculty: **Medicine and Health Science**

Dendritic cells (DC) are efficient and potent antigen-presenting cells in our immune system. The ability of DC to present antigens and stimulate T cells has prompted their application as therapeutic cancer vaccines. The objective of this study was to generate DC from two resources: monocytes and AML blasts. The generated DC were evaluated for their morphology by phase contrast microscopy and May Grunwald Giemsa staining. Viability of cells was determined by trypan blue dye exclusion. Percentage of yields and immunophenotypes were carried out with flow cytometry. The functional capability of DC was also tested in Mixed Leukocyte Reactions and anti-leukaemia cytotoxicity assay. As a result, the generated DC shown typical morphology of those reported and expressed DC surface markers

including CD1a, CD83, CD86, CD80 and HLA-DR. Down regulation of CD14 was also observed for cultured monocytes. In MLR assay, both generated DC elicited strong allo-stimulatory response up to more than 100 fold compared to preculture cells. Mild anti-leukaemia cytotoxicity effect (15%) was also observed from primed effector cells with AML antigen pulsed DC generated from monocytes. These data indicate that DC were successfully generated from the two resources and they were capable of eliciting immune response.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai Memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN, FENOTIPIK DAN ANALISIS FUNGSI SEL DENDRITIK (DC) DARIPADA MONOSIT MANUSIA DAN SEL MIELOID LEUKEMIA AKUT (AML)

Oleh

LIM MOON NIAN

Jun 2004

Pengerusi: **Profesor Seow Heng Fong, Ph.D.**

Fakulti: **Perubatan dan Sains Kesihatan**

Sel dendritik (DC) merupakan sel yang efisien dan penting dalam pemaparan antigen di dalam sistem imun kita. Kebolehan DC untuk memaparkan antigen dan merangsang sel T telah mencungkilkan penggunaan sel ini sebagai vaksin kanser dalam terapiutik. Objektif kajian ini adalah untuk menghasilkan DC daripada dua jenis sumber iaitu monosit dan blas leukemia mieloid akut (AML). DC yang dihasilkan dikaji dari segi morfologi dengan menggunakan mikroskopi berfasa kontras dan pewarnaan May Grumwald Giemsa. Kehidupan sel ditentukan dengan cara pengecualian pewarnaan tripan biru. Peratusan penghasilan dan imunofenotip DC ditentukan dengan cara aliran sitometri. Kebolehan fungsi DC ditentukan dengan asai Reaksi Campuran Leukosit dan sitotoksik anti-leukaemia. Sebagai keputusan, DC yang dihasilkan menunjukkan morfologi yang tipikal sepertimana

yang dilaporkan dan mengekspres pelbagai tanda permukaan DC termasuk CD1a, CD83, CD86, CD80 dan HLA-DR. Penurunan ekspresi CD14 daripada monosit yang telah dikultur juga diperhatikan. Dalam asai Reaksi Campuran Leukosit, kedua-dua DC yang dihasilkan berjaya menjana respons alo-stimulasi yang kuat iaitu lebih daripada 100 kali berbanding dengan sel yang belum dikultur. Kesan sitotoksik anti-leukaemia yang sederhana (15%) diperhatikan daripada sel efektor yang telah dikultur bersamaan dengan DC yang dihasilkan daripada monosit dan telah dipaparkan kepada antigen AML. Semua data di atas menunjukkan bahawa DC telah berjaya dihasilkan daripada dua jenis sumber tersebut dan berfungsi dalam menjana respons imun.

ACKNOWLEDGEMENTS

First and foremost, the author would like to take this opportunity to express her profound to the supervising committee members, Prof. Dr. Seow Heng Fong from Faculty of Medicine and Health Sciences, UPM, Prof. Dr. Cheong Soon Keng and Dr. Leong Chooi Fun from Faculty of Medicine, UKM for their patience, valuable guidance, exhortation, and encouragement throughout the completion of the research. Without their assistance and valuable contribution, this work would have been impossible. I am especially grateful to Majlis Kanser Nasional (MAKNA) for financially support this research.

My appreciations also go out to Madam Siva Gengei K, Mr. Soo Eng Tong and Mr. Lim Jee Hian who always assist me during the course of this project. My appreciation is also extended to former colleagues and friends especially Puan Maha Abdullah, Aini Ardena Mohd. Mustapha, Rachel Mok, Sim Geok Choo, Erica Choong, Fazlina Nordin, Wong Chee Yin, Habsah Aziz and Roslizawati for bringing good memorial time during my course of study. I am also grateful to Dr. Harvindar Kaur Gill from IMR for sharing her experience with me.

Last but not least, I would like to express my heartiest appreciation and thanks to my husband and family members. Thanks for all your understanding and support throughout my studies.

I certify that an Examination Committee met on 3rd June 2004 to conduct the final examination of Lim Moon Nian on her Master of Science thesis entitled "Generation, Phenotyping and Functional Analysis of Dendritic Cells Derived from Human Monocytes and Blasts Cells in Acute Myeloid Leukemia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

DATIN GANASOTHIE DURAISAMY, Ph.D.
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

ABDUL RAHMAN OMAR, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

NOR AMALINA EMRAN, Ph.D.
Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

ABDUL AZIZ BABA, Ph.D.
Professor
School of Medical Sciences
Universiti Sains Malaysia-Kampus Kesihatan
(Independent Examiner)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

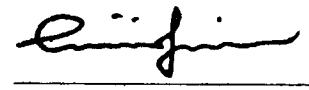
Date: 26 AUG 2004

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follow:

SEOW HENG FONG, Ph.D.
Professor,
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

CHEONG SOON KENG, FASC
Professor,
Faculty of Medicine,
Universiti Kebangsaan Malaysia
(Member)

LEONG CHOOI FUN, MPath
Lecturer,
Faculty of Medicine,
Universiti Kebangsaan Malaysia
(Member)



AINI IDERIS, Ph.D.
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 SEP 2004

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIM MOON NIAN

Date: 20 AUG 2004

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

µL	micro litres
µM	micro molar
ACD-A	anticoagulant citrate dextrose solution formula A
AML	acute myeloid leukaemia
AML-DC	acute myeloid leukaemia blasts derived dendritic cells
BM	bone marrow
BrdU	5-bromo-2'-deoxyuridine
BSA	bovine serum albumin
°C	degree centigrade
CD	cluster of differentiation
cm^2	centimetre square
CO_2	carbon dioxide
CTL	cytotoxic T lymphocytes
D	donor
DC	dendritic cells
$DiOC_{18}$	3, 3'-dioctadecyloxacarbocyanine
DNA	deoxyribonucleic acid
EDTA	ethylene diaminetetra acetic acid
ELISA	enzyme-link immunosorbant assay

E/T	effector/target
F	Formula
FBS	fetal bovine serum
FITC	fluorescein isothiocynate
FSC	forward side scatter
g	gravity force
GM-CSF	granulocyte-macrophage colony-stimulating factor
IL-	interleukine-
LPS	lipopolysaccharide
MFI	mean fluorescent intensity
MGG	May Grunwald Giemsa
mL	millilitres
MNC	mononuclear cells
mAb	monoclonal antibodies
Mo-DC	monocytes derived dendritic cells
ng/mL	nanogram per millilitre
nm	nanometres
P	patient
PBS	phosphate buffer saline

PBMNC	peripheral blood mononuclear cells
PE	phycoerythrin
PerCP	peridinin chrophyll protein
PHA	phytohemagglutinin
PI	propidium iodide
RANK	receptor activator of nuclear factor- [kappa] B
RBC	red blood cells
rpm	rotation per minute
R1	region 1
SCF	stem cell factor
S/R	stimulator/responder
SSC	side scatter
TGF-β	tumour growth factor-β
TNF-α	tumour necrosis factor-α
TRANCE	TNF-related activation-induced cytokine
U/mL	Units per millilitre

CHAPTER 1

INTRODUCTION

1.1 The Importance of DC as Professional Antigen Presenting Cells (APC)

The immune system in mice and human contains a distinct group of APC, called dendritic cells (DC) that are specialized to capture antigens and initiate T cell immunity. DC are named because of their distinctive morphology with numerous cell membrane processes, including spiny dendrites, bulbous pseudopods and lamellipodiae or veils (reviewed by Hart, 1997), as shown in Figure 1. The first DC were reported in the skin by Langerhans in 1868. This was followed by Steiman and Cohn who identified mouse spleen DC in 1973 and initiated a series of experiments that established lymphoid-tissue derived DC as potent stimulators of primary immune responses.

The term "Professional Antigen Presenting Cells" is used to denote cells that have both antigen-presenting and accessory or costimulatory functions (Steinman, 1999). Despite other APC such as B cells and macrophages, DC are more "professional" because of their extraordinary capacity for initiating primary T-lymphocyte responses. This is because in addition to processing antigens to peptides that are presented on MHC, DC also express a plethora of second signals that mediate T-cell binding and costimulation. Most of these second signals are membrane

glycoproteins such as intracellular adhesion molecules (ICAMs; CD50, CD54, CD102), and lymphocyte function associated antigens (LFAa) (CD2, CD11a, CD58), and B7(CD80 and CD86).

1.2 Dendritic Cell Lineages and Nomenclature

Generally, there are two types of DC; one is myeloid-derived DC from myeloid stem cells and lymphoid-derived DC from lymphoid stem cells (Hart, 1997).

Myeloid-derived DC are distributed in a way that maximizes antigen capture and subsequently the binding and activation of specific T cells (Figure 2). They could be found in lymphoid organs, such as lymph node, tonsil, spleen, thymus and mucosa-associated lymphoid tissue. Myeloid-derived DC are represented *in vivo* by Langerhans cells (LC) and interstitial DC. The DC found in epidermis were named Langerhans cells. Most organs except the brain have MHC-II- rich DC within the interstitial spaces that are drained by afferent lymphatics. These DC are known as dermal (interstitial) dendritic cells and those in afferent lymph are recognized as veil cells.

Lymphoid lineage-derived DC have different roles compared to myeloid-lineage derived DC. They play an important role in T cells selection and tolerance in the

thymus. Lymphoid DC are abundant in thymus and T cell area of lymph node where they are known as Interdigitating Cells (IDC). B cell areas of lymph node are rich in another type of cells called the Follicular DC (FDC) that are not originated from the bone marrow (Bachereau and Steinman, 1998). FDC are likely to be stromal or fibroblast cells because they do not express the CD45 molecule that is found on all leukocytes and because they share properties with fibroblasts in culture. FDC retain native antigens as immune complexes for presentation to B cells. Therefore, apart from a coincidence in nomenclature, FDC bear little relation to DC, which are marrow-derived leukocytes that present processed antigens to T cells.

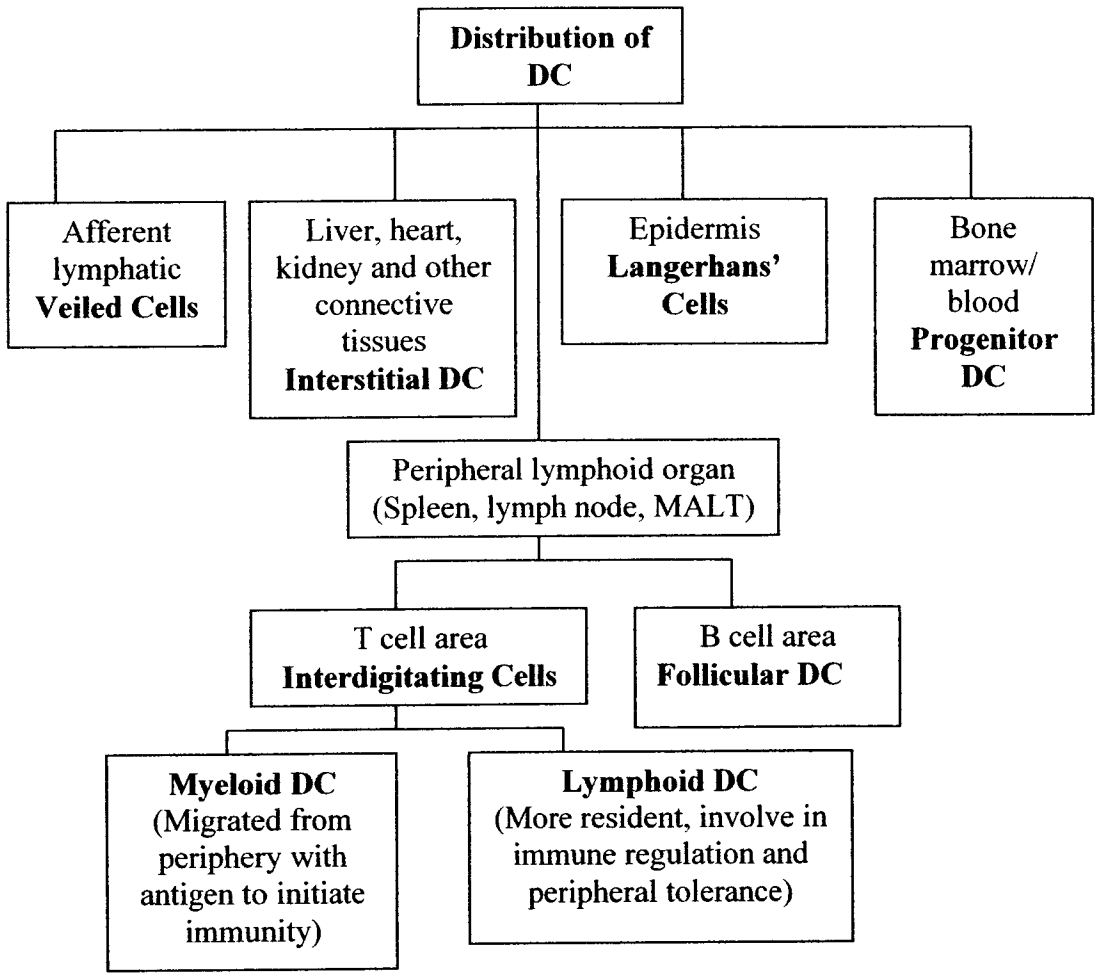


Figure 2: Distribution of DC in human body.

1.3 DC Vaccines for Cancer Immunotherapy

Human tumours express a number of protein antigens that can be recognized by T cells, thus providing potential targets for cancer immunotherapy (Schreiber, 1993). The ability of DC to present antigens and stimulate T cells has prompted their recent application as therapeutic cancer vaccines (Timmerman and Levy, 1999). Isolated DC loaded with tumour antigen *ex vivo* and administered as a cellular vaccine have been found to induce protective and therapeutic anti-tumour immunity in experimental animals (Mayordomo et al., 1995). In pilot clinical trials of DC vaccination for patients with multiple myeloma, melanoma and prostate cancer, induction of anti-tumour immune responses and tumour regressions have been observed (reviewed by Timmerman and Levy, 1999). Additional trials of DC vaccination for a variety of human cancers had been reported, and new methods for targeting tumour antigens to DC also being explored (reviewed by Timmerman and Levy, 1999). Exploitation of the antigen-presenting properties of DC thus offers promise for the development of effective cancer immunotherapies.